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Back to basics and beyond: increasing the level of resistance to Septoria tritici blotch in wheat

Stephen B. Goodwin

USDA-ARS, Crop Production and Pest Control Research Unit, Department of Botany and Plant Pathology, 915 West State Street, Purdue University, West Lafayette, IN 47907-2054, USA. Email: sgoodwin@purdue.edu

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Abstract. Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), is one of the most ubiquitous and important diseases of wheat worldwide. Losses to STB can range from 30 to 50% in disease-conducive climates. Little progress was made in increasing the level of resistance to STB in wheat prior to 1990, due to a variety of factors, including slow growth of the pathogen in culture, long latent period of the disease, the need for specific environmental conditions for infection, and variability in symptom expression, which complicated the scoring of inoculated plants. To identify and map genes for resistance to STB in the wheat genome, to understand how they function in their interactions with the pathogen and, ultimately, to increase the level of resistance so that the disease can be managed without extensive use of fungicides, crosses between parents differing in response to STB were made or obtained from collaborators for the resistance genes *Stb1*, *Stb2*, *Stb3*, *Stb4* and *Stb8*. Plants were grown and inoculated with one or more isolates of *M. graminicola* in a greenhouse. The five targeted resistance genes *Stb1–Stb4* and *Stb8* were mapped to wheat chromosomes 5BL, 3BS, 6DS, 4DS, and 7BL, respectively. All of the genes had at least one linked microsatellite locus, and two of them (*Stb2* and *Stb8*) were mapped between flanking microsatellites. These experiments plus those in other laboratories worldwide have determined the map locations for 12 genes for resistance to STB in wheat during the past 7 years. Most of these genes have associated molecular markers that will be useful for future marker-assisted selection. These analyses were aided by accurate phenotypic analysis, which remains the most difficult part of the process. Technological approaches for improving phenotypic evaluation show promise, including measuring fungal biomass and estimating expression of host genes that are associated with disease resistance by real-time PCR, but they will work better when augmented with improved methods of plant inoculation. Although there is still a great need for more markers, additional mapped genes, and a better understanding of defence responses, recent results now provide the basis for rapid progress in increasing the level of resistance to STB in wheat.

Introduction

Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), is one of the most ubiquitous and important diseases of wheat worldwide (Eyal *et al*. 1987). Losses resulting from STB can range from 30 to 50% in climates conducive to disease development (Eyal *et al*. 1987). Infections on the flag leaves can cause the most severe losses by reducing grain test weight. The disease increased in prominence worldwide after the 1960s with the release of short-statured wheats grown under high-input conditions. This was due to a greater susceptibility of dwarf cultivars (Eyal 1981). Disease incidence also may be higher under minimum tillage practices where crop residue remaining on the soil surface provides higher levels of inoculum.

The genus *Mycosphaerella* contains more than 1000 names and over 500 accepted species of plant pathogens (Corlett 1991). Hosts for species in the genus include almost every major crop, although *M. graminicola* is the only one that is a serious problem on wheat. More than 40 asexual (anamorph) genera are associated with *Mycosphaerella*, some of which are also very large. For example, the anamorph genera *Cercospora* and *Septoria* each contain more than 1000 species infecting a wide diversity of hosts. Together, *Mycosphaerella* and its anamorphs probably comprise the largest group of plant-pathogenic fungi.

M. graminicola has a bipolar, heterothallic mating system with ascospores that are two-celled and forcibly ejected from the ascus at maturity (Kema *et al*. 1996*c*). Following infection, *M. graminicola* produces necrotic lesions filled with asexual fruiting bodies called pycnidia. Pycnidiospores are splash dispersed and provide a means for clonal propagation over short distances (Shaw and Royle 1993). Necrosis presumably is caused by toxic compounds produced by the fungus during intercellular colonisation (Kema *et al*. 1996*d*). However, very little is known about the molecular and genetic mechanisms of disease resistance, and the mechanisms of virulence in *M. graminicola* are only just beginning to be analysed (Cousin *et al*. 2006; Mehrabi *et al*. 2006*a*, 2006*b*).

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The disease cycle of STB probably begins with windborne ascospores produced on stubble remaining from the previous season's crop (Shaw and Royle 1989). The spores are blown to wheat seedlings and establish primary infections. Symptoms of STB consist of small, tan coloured lesions of necrotic tissue that usually are delimited by leaf veins so they appear linear or rectangular. Under favourable conditions, the lesions can cross the boundaries of leaf veins to become irregular or lens shaped. Asexual spores are produced within pycnidia, which form mostly in stomatal cavities and appear as black dots within areas of dead tissue. Symptoms typically appear within 14–21 days after initial infection and there can be many cycles of asexual reproduction during the course of an epidemic. Pycnidiospores spread infections on the same leaf and may be splash dispersed to nearby leaves (Shaw and Royle 1993). It is not clear if ascospores continue to be released during the growing season or if most of the epidemic proceeds by splash dispersal of pycnidiospores. Most likely it is a combination of both, with initial infection of most leaves caused by ascospores and additional spread on the leaf caused by pycnidiospores (Shaw and Royle 1989, 1993). Ascospores are capable of being released from stubble during the growing season (Kema 1996), but whether new infections go through the sexual cycle during the growth cycle of the plant is not known. However, the sexual cycle can be completed within a few weeks under partially controlled conditions (Kema *et al*. 1996*c*). Thus, the production of ascospores from new infections during the course of a growing season seems possible.

Management of the disease is by planting of resistant cultivars when available or, when feasible economically, by spraying fungicides. For example, in Europe more than 70% of the fungicides applied to wheat are to combat this disease. STB caused by *M. graminicola* is one of the top two or three diseases in most wheat-growing regions worldwide, including Europe, North America, South America, and Australia (Eyal *et al*. 1987).

Populations of *M. graminicola* are highly variable for molecular markers (McDonald and Martinez 1990*a*, 1990*b*, 1991*a*, 1991*b*), and analyses of restriction fragment length polymorphism (RFLP) data revealed very high levels of genetic diversity within fields. For example, among 711 isolates analysed from one field in Oregon, USA, 654 distinct genotypes were identified (Boeger *et al*. 1993; Zhan *et al*. 2003). Identical clones usually occurred only on the same leaf; samples from adjacent leaves in the same field usually were genetically distinct. This is consistent with a high level of sexual recombination and supports the epidemiological studies, which implicated ascospores as primary inoculum (Shaw and Royle 1989). The high level of genotypic diversity also may indicate that ascospores are produced during the course of a season and may provide secondary as well as primary inoculum.

High levels of within-field diversity are not limited to Oregon, but are characteristic of *M. graminicola* populations worldwide. Among 15 populations from throughout the world, the mean gene diversity over seven or eight RFLP loci was 0.35 with a mean of 18 alleles per locus (Zhan *et al*. 2003). Effective population sizes must be extremely large to maintain such a high level of allelic diversity within populations. With large population sizes, genetic drift will be minimal, so we would expect the genetic composition of populations to change only slowly over time, unless affected by strong selection or migration. Strong selection is unlikely to act on the molecular markers analysed in most studies of genetic variation within populations of *M. graminicola*, although it might act on virulence and tightly linked molecular markers.

In contrast to the molecular markers, much less is known about variability for pathogenicity within populations. Some early work failed to identify strong cultivar–isolate interactions indicating that specificity was lacking (Van Ginkel and Scharen 1987, 1988*a*, 1988*b*). Other work with large numbers of cultivars and isolates identified many specific interactions (Eyal *et al*. 1973, 1985; Eyal and Levy 1987; Kema *et al*. 1996*a*, 1996*b*). Recent analyses of the genetics of the host and the pathogen revealed clear interactions between a resistance gene in the host (Brading *et al*. 2002) and corresponding virulence in the pathogen (Kema *et al*. 2000), confirming the existence of gene-for-gene interactions at least for some cultivar–isolate pairs. However, much of the resistance against *M. graminicola* in wheat clearly is quantitative and possibly non-specific, and both appear to be important to conferring full resistance in the field (Arraiano and Brown 2006).

State of the art in 1995

Despite the frequent occurrence and high economic significance of STB, most of the more commonly grown wheat cultivars seem to contain little if any effective resistance. This is surprising given the long history of problems associated with this disease. Lack of progress is due to a variety of factors, including slow growth of the pathogen in culture, long latent period of the disease, the need for specific environmental conditions for infection, and variability in symptom expression that complicates the scoring of inoculated plants. These difficulties probably explain the long delay from their first identification until any STB genes were mapped genetically.

Early work on the genetics of resistance to STB in wheat indicated that it could be quantitative or qualitative (Narvaez and Caldwell 1957). The first gene to be analysed thoroughly, named *Stb1* by Wilson (1985), was identified in the wheat cultivar Bulgaria 88 by Rillo and Caldwell (1966). Genes *Stb2* and *Stb3* were identified in Australia by Wilson (1985), and were derived originally from cvv. Veranopolis and Israel 493, respectively. Discovery of the *Stb4* gene in cv. Tadorna by Somasco *et al*. occurred in 1996 (Somasco *et al*. 1996).

Only two of these genes seem to have been used in wheat improvement. The *Stb1* gene was incorporated into the Indiana soft red winter cvv. Oasis and Sullivan by Patterson *et al*. (1975, 1979), and this gene provided long-lasting resistance to wheat in Indiana and other parts of the Midwestern US. The *Stb4* gene was bred initially into the California cv. Tadinia and was effective for ~15 years (Somasco *et al.* 1996). However, this resistance broke down by 2000 (Jackson *et al*. 2000) and cultivars that contain it now are considered susceptible. Thus, when the author first began to work on STB during 1995, three single, dominant genes for resistance had been identified in wheat, but none had been mapped and no markers were available for easy transfer of the genes to improved cultivars by marker-assisted selection. This remained true even after the *Stb4* gene was published in 1996. Clearly, many more genes were needed to confer a lasting resistance and much more genetic work was required

for a comprehensive picture of resistance and of host-pathogen interactions.

Rapid progress in genetic mapping

Fortunately, genetic mapping of STB resistance genes progressed rapidly after 1995 and we now have 12 genes that have been mapped, with one or more associated molecular markers (Table 1). *Stb5* was discovered and mapped in 2001 (Arraiano *et al*. 2001*b*), followed quickly by *Stb6* in 2002 (Brading *et al*. 2002), *Stb7* (McCartney *et al*. 2003) and *Stb8* (Adhikari *et al*. 2003) in 2003, *Stb10*, *Stb11* and *Stb12* in 2005 (Chartrain *et al*. 2005*a*, 2005*b*), and *Stb15* in 2007 (Arraiano *et al*. 2007). The previously named genes *Stb1*, *Stb2*, *Stb3* and *Stb4* were all mapped in 2004 (Adhikari *et al*. 2004*b*, 2004*c*, 2004*d*). Although it took 35 years from identification and naming of the first STB gene until the first one was mapped, 11 more followed in the succeeding 6 years, giving us now a good collection of named and mapped genes for resistance to this disease. These genes are distributed on 10 wheat chromosomes, occurring equally on the A, B and D genomes with four genes apiece, and on all seven homologous groups except for Group 2. Two possible clusters occur on the long arm of chromosome 4A (*Stb7* and *Stb12*) and on chromosome 7D near the centromere (*Stb4* and *Stb5*) (Table 1).

The next steps

Now that the initial flurry of mapping has been completed, we can look forward to a new era of marker-assisted selection. However, before that can occur the available markers must be augmented and improved. Currently, only three resistance loci (*Stb2*, *Stb8* and *Stb12*) have flanking microsatellite or simple-sequence repeat (SSR) markers (Table 1). For the others, no flanking marker is available or it is of a type that is more difficult to score. Good flanking markers are essential to ensure that the resistance gene is not lost during the process of marker-assisted selection. A single crossover between the marker and the resistance locus will eliminate their association, but will be detected and avoided with a closely linked flanking marker. Losing the association between the resistance gene and two flanking markers is possible theoretically by a double crossover, but these are very rare and unlikely to be a factor in a program of marker-assisted selection.

Another potential problem with the available markers is that their level of polymorphism may be low, limiting their utility in additional crosses. Markers linked to the *Stb1* and *Stb2* loci had a high level of polymorphism when tested on additional cultivars (Adhikari *et al*. 2004*c*, 2004*d*), but for markers linked to most of the other genes, the level of polymorphism was lower or was not tested. It is also desirable to have markers that are linked more closely than those identified thus far, both for marker-assisted selection and for possible map-based cloning of the genes in the future. Now that the map locations are known, it should be a straightforward process to identify additional linked markers by screening those known to lie in nearby locations for polymorphism on the parents of the available mapping populations.

Improvement of the markers themselves may also be possible. The primers that identify many of the microsatellite loci amplify more than one band in many wheat cultivars. These bands often are homologous microsatellites on one or more of the other two genomes of hexaploid wheat, but can also arise from spurious binding of the primers at unrelated loci. Cloning and sequencing of the correct locus can allow the primers to be extended and possibly made more specific. This approach is not warranted for every linked marker, but is worthwhile for those that are

Table 1. Published chromosomal locations and molecular markers associated with 12 named genes for resistance to Septoria tritici blotch in wheat

Gene	Original source cultivar	Chromosomal location	Nearest molecular marker[A]	Nearest flanking marker	Reference
Stb1	Bulgaria 88	5BL	SSR, 2.8 cM	AFLP, 8.4 cM	Adhikari *et al*. (2004*d*)
Stb2	Veranopolis	3BS	SSR, 0.9 cM	SSR, 3.7 cM	Adhikari *et al*. (2004*c*)
Stb3	Israel 493	6DS[B]	SSR, 3.0 cM	–[C]	Adhikari *et al*. (2004*c*)
Stb4	Tadorna	7DS	SSR, 0.7 cM	AFLP, 4.0 cM	Adhikari *et al*. (2004*b*)
Stb5	Synthetic 6x	7DS	SSR, 7.2 cM	Rc3[D], 6.6. cM	Arraiano *et al*. (2001*b*)
Stb6	Flame, Hereward	3AS	SSR, 2.0 cM	SSR[E]	Brading *et al*. (2002)
Stb7	Estanzuela Federal (ST6)	4AL	SSR, 0.3 cM	–[C]	McCartney *et al*. (2003)
Stb8	Synthetic W7984	7BL	SSR, 3.5 cM	SSR, 5.3 cM	Adhikari *et al*. (2003)
Stb10	Kavkaz-K4500 L.6.A.4	1D	SSR[F]	–[C]	Chartrain *et al*. (2005*a*)
Stb11	TE911	1BS	SSR[F]	–[C]	Chartrain *et al*. (2005*b*)
Stb12	Kavkaz-K4500 L.6.A.4	4AL	SSR[F]	SSR[F]	Chartrain *et al*. (2005*a*)
Stb15	Arina	6AS	RFLP[F]	–[C]	Arraiano *et al*. (2007)

[A]Distances in centiMorgans of the most closely linked molecular markers are indicated. AFLP, amplified fragment length polymorphism; RFLP, restriction fragment length polymorphism; SSR, simple-sequence repeat or microsatellite.

[B]Recent attempts to confirm this location were not successful. It is most likely this gene was mapped incorrectly and instead it appears to be located on chromosome 7AS (S. B. Goodwin, J. R. Cavaletto, J. Dubcovsky, unpubl. data).

[C]No flanking marker found.

[D]This is the morphological marker for red coleoptile. No flanking molecular marker is available.

[E]Tentative positioning according to Brading *et al*. (2002) so the map location and distance are uncertain.

[F]Mapped by quantitative trait loci analysis so exact map distances are not available.

closely linked to an important resistance locus and also are highly polymorphic among many wheat cultivars.

In addition to a great need for additional linked markers, the published map locations should be verified in additional mapping populations. Most of these genes were mapped with a single, often relatively small, progeny set, so the map locations may be imprecise or inaccurate. Mapping in additional crosses with larger numbers of progeny is essential to confirm and refine the published map locations. An example of this is the *Stb3* locus, which was mapped to chromosome 6DS with only a single SSR marker (Adhikari *et al*. 2004*c*). Our numerous attempts to find additional markers linked to this locus failed; all markers we tested subsequently on chromosome 6DS that were polymorphic between the parents of the original mapping population were not linked to *Stb3*. After reexamining the data, it appears that the previous linkage was incorrect. Screening of a large number of additional markers on other chromosomes finally identified a linkage block containing *Stb3* on chromosome 7AS (S. B. Goodwin *et al*., unpubl. data). Testing of additional progeny sets to verify the new map location is under way.

Once the map locations have been verified in other crosses and refined with additional markers, the next step will be to combine loci from different sources into single linkage blocks for enhanced resistance to the same or different pathogens. For example, resistance genes *Stb7* and *Stb12* are both on the long arm of chromosome 4A and, if not allelic, could be combined into a single linkage block. This could also be attempted for *Stb4* and *Stb5* on 7DS, and for any new STB genes that are mapped near to those that have been mapped previously. These endeavours will be aided immensely by the availability of a large number of closely linked molecular markers.

In addition to combining resistance genes at nearby loci into chromosome segments with multiple specificities, the markers can aid pyramiding of STB resistance genes at multiple unlinked loci. Recent genetic dissection of highly resistant wheat cultivars revealed that they often contain multiple resistance genes, indicating that gene pyramids may provide effective resistance against STB in the field (Chartrain *et al*. 2004, 2005*a*, 2005*b*). However, even these highly resistant lines are overcome by occasional isolates of *M. graminicola* in laboratory tests, so such pyramided resistance may break down in the future. For this reason there is a continuing need to identify new and better sources of resistance.

A similar use for molecular markers will be to combine resistance to multiple diseases and pests. This is being attempted already for the *Stb2* gene on chromosome 3BS. In addition to *Stb2*, this chromosome arm also contains gene *Sr2* for resistance against stem rust (Spielmeyer *et al*. 2003), and major quantitative trait loci (QTL) for resistance to Stagonospora nodorum blotch (Schnurbusch *et al*. 2003) and Fusarium head scab (Liu and Anderson 2003). All of these resistances originated in different cultivars of wheat. Attempts are under way to combine these in coupling into a single linkage block with good resistance to all four diseases. Similar map locations between other STB resistance genes and genes for resistance to various pests and pathogens also exist and could be exploited to develop linkage blocks with multiple resistances.

Back to basics: phenotyping the STB resistance response

After all of the progress made during the past dozen years, phenotyping of the host response is still the most variable and uncertain part of the process. Difficulties in phenotypic scoring provided the impetus driving the identification of linked molecular markers. However, several STB resistance genes show relatively weak phenotypes, and many have been mapped as QTL rather than qualitative traits (Arraiano *et al*. 2007). Problems in phenotypic analysis derive from the long latent period of the disease and environmental influences on symptom development. Symptoms usually do not begin to become visible until at least 14–18 days after inoculation, and full symptom expression often requires between 21 and 28 days or more. Symptoms often vary among susceptible plants, presumably due to differences in microenvironment during the inoculation and infection process. This variation requires extensive replication of plants and tests to be certain that a phenotype has been assigned correctly.

Many approaches have been taken to minimise these problems, including tests of seedling plants in growth chambers (Adhikari *et al*. 2004*d*) or air-conditioned greenhouses (Kema *et al*. 1996*a*), detached-leaf tests (Arraiano *et al*. 2001*a*), or inoculation of newly expanded flag leaves to maximise the time for symptom expression before leaf senescence (Adhikari *et al*. 2003). Although these approaches have proven to be very useful, they all have their drawbacks: throughput may be limited in greenhouses or growth chambers; seedling tests may not indicate resistance of adult plants and *vice versa*; and much time is required to grow plants to near maturity for flag-leaf inoculations. For these reasons, we explored alternative methods of quantifying resistance to STB in wheat plants.

Our first approach was to use real-time PCR to measure fungal biomass. Although symptoms typically are not expressed until 18–20 days after inoculation, our hypothesis was that an increase in fungal biomass would occur in susceptible plants much earlier, hopefully by 3–5 days after inoculation. This was tested by inoculating two resistant and two susceptible cultivars of wheat with an isolate of *M. graminicola* and collecting leaves about every 3 days for 27 days after inoculation. Fungal biomass was estimated with primers to the β-tubulin gene of the fungus by real-time PCR.

Unfortunately, the results were not what we expected. Instead of a rapid increase of fungal biomass in the susceptible cultivars, biomass stayed low in all four cultivars until ∼14 days after inoculation, when it increased exponentially in the two susceptible cultivars but remained flat or decreased in those that were resistant (Adhikari *et al*. 2004*a*). Although it was easy to distinguish resistant from susceptible cultivars by 16+ days after inoculation, this did not provide much of a benefit over scoring the plants for disease, especially given the high time and cost required for real-time PCR. Levels of fungal biomass were significantly higher in the susceptible cultivars at earlier time points, but the magnitude of the difference was much lower and would require several replications for reliable discrimination. Real-time PCR of *M. graminicola* biomass was able to differentiate resistant from susceptible recombinant-inbred lines, but the technique requires too much replication to be useful in most large-scale applications.

Our second approach was to use real-time PCR to measure plant gene expression. Previous experiments showed that resistant plants responded to contact with the pathogen with expression of certain genes much stronger than did susceptible plants (Ray *et al*. 2003; Adhikari *et al*. 2007). Some of the genes tested were pathogenesis-related proteins that were known to be associated with resistance responses in plants, while others had not been implicated previously as possibly being associated with or involved in defence responses. Susceptible plants responded less strongly or not at all as measured by gene transcription in relation to a water-inoculated control by real-time PCR (Adhikari *et al*. 2007).

Tests of gene expression in segregating recombinant-inbred lines (RILs) by real-time PCR showed that expression was greater in all of the resistant lines than in any of those that were susceptible at 12 h after inoculation. This time point was chosen for analysis because it showed the largest difference between the parents of the RIL population during the 27-day analysis and would provide a quick identification of resistant cf. susceptible plants. Although it provided a clear separation between resistant and susceptible lines, the magnitude of the difference was not as large as desirable for routine use. This approach has much potential and can give results much sooner than scoring symptoms, but will need to be refined, perhaps by identifying a gene with greater differential expression or by sampling at different time points. Testing a high proportion of wheat genes for differential expression could be conducted, for example, with the Affymetrix wheat genome array (http://www.affymetrix.com/products/arrays/specific/wheat.affx, verified 12 September 2007) to identify likely candidates for testing by real-time PCR. Another caveat for this approach is that there was variation in the magnitude and timing of gene expression among susceptible and resistant cultivars so it may be necessary to use a different marker gene for each resistance gene analysed.

Although the biotechnological approaches have much potential and will be very useful in specific situations, particularly in genetic analyses in the laboratory, they probably will be of less use in classical plant breeding programs due to the cost and complexity of the assays. This again brings us back to the basics: accurate phenotypic scoring is still the most time consuming and difficult part of the process, yet it is essential for future progress. Part of the problem with inoculation assays in the greenhouse is the need for plants to be kept at high humidity for three days after inoculation to encourage infection. In our greenhouses in Indiana, this is accomplished by enclosing the inoculated plants in small chambers made by attaching plastic sheeting to adjustable frames of polyvinyl chloride tubing. These chambers are essential to maintain adequate humidity for inoculation, yet on sunny days can become too hot for optimal growth of the pathogen leading to failed inoculation experiments.

An alternative inoculation method that we are testing currently is to inject spores directly into the whorls of developing leaves. The enclosed whorls of leaves provide the humidity and other favourable conditions required for successful infection and eliminate the need to place the plants in inoculation chambers. Symptoms become evident after the leaves develop and expand fully; the point of inoculation is identified by a small puncture wound. Preliminary tests of this approach look very promising, especially with very strong resistance genes such as *Stb2*, and symptoms were evident much earlier than with our usual greenhouse testing. Further experiments to test this approach on segregating progeny lines are in progress.

What about the pathogen?

Progress in understanding the genetic basis of virulence in the pathogen has been occurring in parallel to the increase in our knowledge about the genetics of resistance in the host. Since the discovery of its teleomorph in 1972 (Sanderson 1972), little progress was made on the genetics of the pathogen until Kema *et al*. (1996*c*) published their analysis of the first controlled cross between isolates of *M. graminicola*. This groundbreaking work stimulated additional research that led to a genetic map in 2002 (Kema *et al*. 2002), and culminated with sequencing the genome of *M. graminicola* by the USA Department of Energy's Joint Genome Institute in 2006 (http://genome.jgi-psf.org/Mycgr1/Mycgr1.home.html, verified 12 September 2007). The initial release was an 8.9 × draft sequence, but this will be followed by a finished sequence containing few if any gaps – hopefully later this year. Thus, we will have a complete accounting of every base in the genome of *M. graminicola* isolate IPO323 only 11 years after the first published report of a genetic cross.

The genome sequence is a stepping stone to a more complete understanding of the interactions between *M. graminicola* and wheat. Two gene chip arrays are planned based on the genomic sequence. The first is a tiling array with 50-mer probes spaced every 100 bases over the entire genome (G. H. J. Kema, pers. comm.). The second will be an expression array containing most or all of the genes identified in the genomic sequence. This array will complement the tiling array by facilitating accurate estimation of gene expression under different treatments. These arrays, combined with the wheat genome array already in existence, will provide unprecedented information about the expression of host and pathogen genes during their interactions leading to resistance or disease. The anticipated gene chip experiments should generate testable hypotheses to keep septoria researchers productively busy for the next decade and more.

Conclusions

In a 1990 review, Nelson and Marshall wrote, 'Compared with other wheat diseases, such as leaf or stem rust, little progress in host plant resistance has been accomplished with the septoria diseases' (Nelson and Marshall 1990). Fortunately, this statement is no longer true. Instead, the rapid progress made during the past 17 years has brought us to the point where we now have a hope of managing STB by increasing the level of resistance. However, with the high genetic variability of the pathogen it seems likely that most resistance genes will not last long so there will be a continual need to identify new strategies for effective disease management. During the coming decade our understanding of host–pathogen interactions will improve dramatically, and might identify new weaknesses in the pathogen that could allow for better uses of resistance, or for improved methods of chemical control. With the recent progress in genetic mapping, it also seems likely that one or more STB resistance

genes will be cloned during the next 10 years, and this might provide a path for increasing resistance through biotechnology. All of these will depend on maintaining and strengthening the basics of phenotypic analysis.

References

Adhikari TB, Anderson JM, Goodwin SB (2003) Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola. Phytopathology* **93**, 1158–1164. doi: 10.1094/PHYTO.2003.93.9.1158

Adhikari TB, Balaji B, Breeden J, Anderson JM, Goodwin SB (2004*a*) Quantification of *Mycosphaerella graminicola* in wheat by real-time PCR. *Phytopathology* **94**, S2. [Abstract].

Adhikari TB, Cavaletto JR, Dubcovsky J, Gieco J, Schlatter AR, Goodwin SB (2004*b*) Molecular mapping of the *Stb4* gene for resistance to septoria tritici blotch in wheat. *Phytopathology* **94**, 1198–1206. doi: 10.1094/PHYTO.2004.94.11.1198

Adhikari TB, Wallwork H, Goodwin SB (2004*c*) Microsatellite markers linked to the *Stb2* and *Stb3* genes for resistance to Septoria tritici blotch in wheat. *Crop Science* **44**, 1403–1411.

Adhikari TB, Yang X, Cavaletto JR, Hu X, Buechley G, Ohm HW, Shaner G, Goodwin SB (2004*d*) Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. *Theoretical and Applied Genetics* **109**, 944–953. doi: 10.1007/s00122-004-1709-6

Adhikari TB, Balaji B, Breeden JD, Goodwin SB (2007) Resistance of wheat to *Mycosphaerella graminicola* involves early and late peaks of gene expression. *Physiological and Molecular Plant Pathology*, in press.

Arraiano LS, Brown JKM (2006) Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. *Plant Pathology* **55**, 726–738. doi: 10.1111/j.1365-3059.2006.01444.x

Arraiano LS, Brading PA, Brown JKM (2001*a*) A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. *Plant Pathology* **50**, 339–346. doi: 10.1046/j.1365-3059.2001.00570.x

Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM (2001*b*) Chromosomal location of a gene for resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. *Theoretical and Applied Genetics* **103**, 758–764. doi: 10.1007/s001220100668

Arraiano LS, Chartrain L, Bossolini E, Slatter HN, Keller B, Brown JKM (2007) A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola. Plant Pathology* **56**, 73–78. doi: 10.1111/j.1365-3059.2006.01499.x

Boeger JM, Chen RS, McDonald BA (1993) Gene flow between geographic populations of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. *Phytopathology* **83**, 1148–1154. doi: 10.1094/Phyto-83-1148

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM (2002) A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the Septoria tritici blotch pathogen. *Phytopathology* **92**, 439–445. doi: 10.1094/PHYTO.2002.92.4.439

Chartrain L, Brading PA, Makepeace JC, Brown JKM (2004) Sources of resistance to septoria tritici blotch and implications for wheat breeding. *Plant Pathology* **53**, 454–460. doi: 10.1111/j.1365-3059.2004.01052.x

Chartrain L, Berry ST, Brown JKM (2005*a*) Resistance of wheat line Kavkaz-K4500 L.6.A.4 to septoria tritici blotch controlled by isolate-specific resistance genes. *Phytopathology* **95**, 664–671. doi: 10.1094/PHYTO-95-0664

Chartrain L, Joaquim P, Berry ST, Arraiano LS, Azanza F, Brown JKM (2005*b*) Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. *Theoretical and Applied Genetics* **110**, 1138–1144. doi: 10.1007/s00122-005-1945-4

Corlett M (1991) An annotated list of the published names in *Mycosphaerella* and *Sphaerella. Mycological Memoirs* **18**, 1–328.

Cousin A, Mehrabi R, Guilleroux M, Dufresne M, Van der Lee T, Waalwijk C, Langin T, Kema GHJ (2006) The MAP kinase-encoding gene *MgFus3* of the non-appressorium phytopathogen *Mycosphaerella graminicola* is required for penetration and *in vitro* pycnidia formation. *Molecular Plant Pathology* **7**, 269–278. doi: 10.1111/j.1364-3703.2006.00337.x

Eyal Z (1981) Integrated control of Septoria diseases of wheat. *Plant Disease* **65**, 763–768.

Eyal Z, Levy E (1987) Variations in pathogenicity patterns of *Mycosphaerella graminicola* within *Triticum* spp. in Israel. *Euphytica* **36**, 237–250. doi: 10.1007/BF00730670

Eyal Z, Amiri Z, Wahl I (1973) Physiologic specialization of *Septoria tritici. Phytopathology* **63**, 1087–1091.

Eyal Z, Scharen AL, Huffman MD, Prescott JM (1985) Global insights into virulence frequencies of *Mycosphaerella graminicola. Phytopathology* **75**, 1456–1462.

Eyal Z, Scharen AL, Prescott JM, Van Ginkel M (1987) 'The Septoria diseases of wheat: concepts and methods of disease management.' (CIMMYT: Mexico)

Jackson LF, Dubcovsky J, Gallagher LW, Wennig RL, Heaton J, *et al*. (2000) 2000 Regional barley and common and durum wheat performance tests in California. *Agronomy Progress Report* **272**, 1–56.

Kema GHJ (1996) *Mycosphaerella graminicola* on wheat: genetic variation and histopathology. PhD Thesis, Wageningen Agricultural University, The Netherlands.

Kema GHJ, Annone JG, Sayoud R, van Silfhout C, van Ginkel M, de Bree J (1996*a*) Genetic variation for virulence and resistance in the wheat – *Mycosphaerella graminicola* pathosystem. I. Interactions between pathogen isolates and host cultivars. *Phytopathology* **86**, 200–212. doi: 10.1094/Phyto-86-200

Kema GHJ, Sayoud R, Annone JG, Van Silfhout CH (1996*b*) Genetic variation for virulence and resistance in the wheat – *Mycosphaerella graminicola* pathosystem. II. Analysis of interactions between pathogen isolates and host cultivars. *Phytopathology* **86**, 213–220. doi: 10.1094/Phyto-86-213

Kema GHJ, Verstappen ECP, Todorova M, Waalwijk C (1996*c*) Successful crosses and molecular tetrad and progeny analyses demonstrate heterothallism in *Mycosphaerella graminicola. Current Genetics* **30**, 251–258. doi: 10.1007/s002940050129

Kema GHJ, Yu DZ, Rijkenberg FHJ, Shaw MW, Baayen RP (1996*d*) Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. *Phytopathology* **86**, 777–786. doi: 10.1094/Phyto-86-777

Kema GHJ, Verstappen ECP, Waalwijk C (2000) Avirulence in the wheat septoria tritici leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. *Molecular Plant-Microbe Interactions* **13**, 1375–1379. doi: 10.1094/MPMI.2000.13.12.1375

Kema GHJ, Goodwin SB, Hamza S, Verstappen ECP, Cavaletto JR, Van der Lee TAJ, Hagenaar-de Weerdt M, Bonants PJM, Waalwijk C (2002) A combined AFLP and RAPD genetic linkage map of *Mycosphaerella graminicola*, the septoria tritici leaf blotch pathogen of wheat. *Genetics* **161**, 1497–1505.

Liu S, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. *Crop Science* **43**, 760–766.

McCartney CA, Brûlé-Babel AL, Lamari L, Somers DJ (2003) Chromosomal location of a race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. *Theoretical and Applied Genetics* **107**, 1181–1186. doi: 10.1007/s00122-003-1359-0

McDonald BA, Martinez JP (1990*a*) DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. *Phytopathology* **80**, 1368–1373.

McDonald BA, Martinez JP (1990*b*) Restriction fragment length polymorphisms in *Septoria tritici* occur at a high frequency. *Current Genetics* **17**, 133–138. doi: 10.1007/BF00312858

McDonald BA, Martinez JP (1991*a*) Chromosome length polymorphisms in a *Septoria tritici* population. *Current Genetics* **19**, 265–271. doi: 10.1007/BF00355053

McDonald BA, Martinez JP (1991*b*) DNA fingerprinting of the plant pathogenic fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*). *Experimental Mycology* **15**, 146–158. doi: 10.1016/0147-5975(91)90015-6

Mehrabi R, van der Lee T, Waalwijk C, Kema GHJ (2006*a*) *MgSlt2*, a cellular integrity MAP kinase gene of the fungal wheat pathogen *Mycosphaerella graminicola*, is dispensable for penetration but essential for invasive growth. *Molecular Plant-Microbe Interactions* **19**, 389–398. doi: 10.1094/MPMI-19-0389

Mehrabi R, Zwiers LH, de Waard MA, Kema GHJ (2006*b*) *MgHog1* regulates dimorphism and pathogenicity in the fungal wheat pathogen *Mycosphaerella graminicola. Molecular Plant-Microbe Interactions* **19**, 1262–1269. doi: 10.1094/MPMI-19-1262

Narvaez I, Caldwell RM (1957) Inheritance of resistance to leaf blotch of wheat caused by *Septoria tritici. Phytopathology* **47**, 529–530. [Abstract].

Nelson LR, Marshall D (1990) Breeding for resistance to *Septoria nodorum* and *Septoria tritici. Advances in Agronomy* **44**, 257–277.

Patterson FL, Roberts JJ, Finney RE, Shaner GE, Gallun RL, Ohm HW (1975) Registration of Oasis wheat. *Crop Science* **15**, 736–737.

Patterson FL, Shaner GE, Huber DM, Ohm HW, Finney RE, Gallun RL, Roberts JJ (1979) Registration of Sullivan wheat. *Crop Science* **19**, 297.

Ray S, Anderson JM, Urmeev FI, Goodwin SB (2003) Rapid induction of a protein disulfide isomerase and defense-related genes in wheat in response to the hemibiotrophic fungal pathogen *Mycosphaerella graminicola. Plant Molecular Biology* **53**, 741–754. doi: 10.1023/B:PLAN.0000019120.74610.52

Rillo AO, Caldwell RM (1966) Inheritance of resistance to *Septoria tritici* in *Triticum aestivum* subsp. *vulgare* 'Bulgaria 88'. *Phytopathology* **56**, 897. [Abstract].

Sanderson FR (1972) A *Mycosphaerella* species as the ascogenous state of *Septoria tritici* Rob. and Desm. *New Zealand Journal of Botany* **10**, 707–710.

Schnurbusch T, Paillard S, Fossati D, Messmer M, Schachermayer G, Winzeler M, Keller B (2003) Detection of QTLs for Stagonospora glume blotch resistance in Swiss winter wheat. *Theoretical and Applied Genetics* **107**, 1226–1234. doi: 10.1007/s00122-003-1372-3

Shaw MW, Royle DJ (1989) Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola*) infections in winter wheat crops in the UK. *Plant Pathology* **38**, 35–43. doi: 10.1111/j.1365-3059.1989.tb01425.x

Shaw MW, Royle DJ (1993) Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. *Plant Pathology* **42**, 882–899. doi: 10.1111/j.1365-3059.1993.tb02674.x

Somasco OA, Qualset CO, Gilchrist DG (1996) Single-gene resistance to *Septoria tritici* blotch in the spring wheat cultivar 'Tadinia'. *Plant Breeding* **115**, 261–267. doi: 10.1111/j.1439-0523.1996.tb00914.x

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). *Crop Science* **43**, 333–336.

Van Ginkel M, Scharen AL (1987) Generation mean analysis and heritabilities of resistance to *Septoria tritici* in durum wheat. *Phytopathology* **77**, 1629–1633.

Van Ginkel M, Scharen AL (1988*a*) Diallel analysis of resistance to *Septoria tritici* isolates in durum wheat. *Euphytica* **38**, 31–37. doi: 10.1007/BF00024808

Van Ginkel M, Scharen AL (1988*b*) Host-pathogen relationships of wheat and *Septoria tritici. Phytopathology* **78**, 762–766.

Wilson RE (1985) Inheritance of resistance to *Septoria tritici* in wheat. In 'Septoria of cereals – Proceedings of the second international Septoria workshop, 2–4 August 1983, Bozeman, Montana, USA'. (Ed. AL Scharen) pp. 33–35.

Zhan J, Pettway RE, McDonald BA (2003) The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology* **38**, 286–297. doi: 10.1016/S1087-1845(02)00538-8

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